Identification and Classification of Non-Coding RNA using Computational Techniques

SHORT SYNOPSIS

Submitted in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

By

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ABSTRACT

According to the central dogma of molecular biology, DNA, which contains the cell's genetic information, is first transcribed into RNA and then translated into proteins. But approximately 60-70% of DNA is transcribed into RNA, and approximately 1-5% of the total RNA is translated into protein. The remaining 95-99% of untranslated RNAs are known as non-coding RNAs. These ncRNAs are involved in many biological processes in humans. Transfer RNA (tRNA) and ribosomal RNA (rRNA) are involved in the protein synthesis. MicroRNAs (miRNAs) regulate gene expression. Small nucleolar RNAs (snoRNAs) are involved in RNA processing and modifications. Small interfering RNAs (siRNAs) protect against viruses. ncRNAs also play a role in cell differentiation and development. ncRNAs are also linked to many diseases, such as cancer, heart disease, and Alzheimer's disease. Owing to the progress in next-generation sequencing (NGS), a large number of sequences have been generated, which makes the processing and analysis of these sequences difficult and time-consuming in the laboratory. There is a need for computational techniques that analyze the sequences and produce some relevant results. In this study, we will apply computational techniques based on machine and deep learning to identify and classify ncRNAs in the human genome.

Keywords: Non-coding RNAs, next-generation sequencing, deep learning, machine learning.

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1. Introduction

1.1 Deoxyribonucleic Acid (DNA)

DNA [1,2,3] is a fundamental organic molecule that contains information and instructions essential for protein synthesis. It is present in most cells of living organisms. DNA is essential for reproduction because it enables the flow of genetic information from parents to their children. A DNA molecule is composed of two strands that complement each other to form a double-helix structure. These strands are connected by hydrogen bonds between specific base pairs. DNA comprises four nucleotide bases: adenine (A), thymine (T), guanine (G), and cytosine (C), in which adenine binds with thymine and guanine with cytosine using covalent bonds.

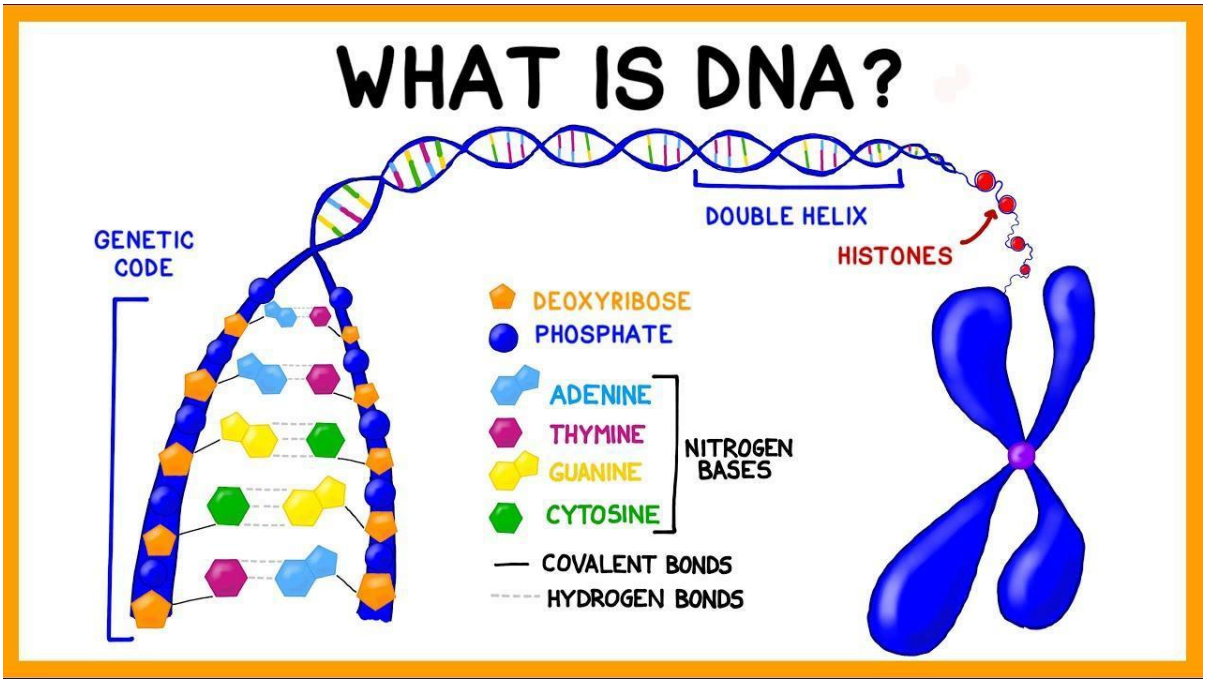

Figure 1: DNA with its building blocks [61]

1.2 Ribonucleic Acid (RNA)

Ribonucleic acid (RNA) [4,5,6] acts as a messenger that copies and translates the genetic code present in DNA to the ribosome. It is a linear polymer of nucleotides, consisting of ribose sugar, a phosphate group, and a nitrogenous base. The nitrogenous bases present in RNA are adenine (A), guanine (G), cytosine (C), and uracil (U). Therefore, uracil is present in RNA instead of thymine, which is present in DNA.

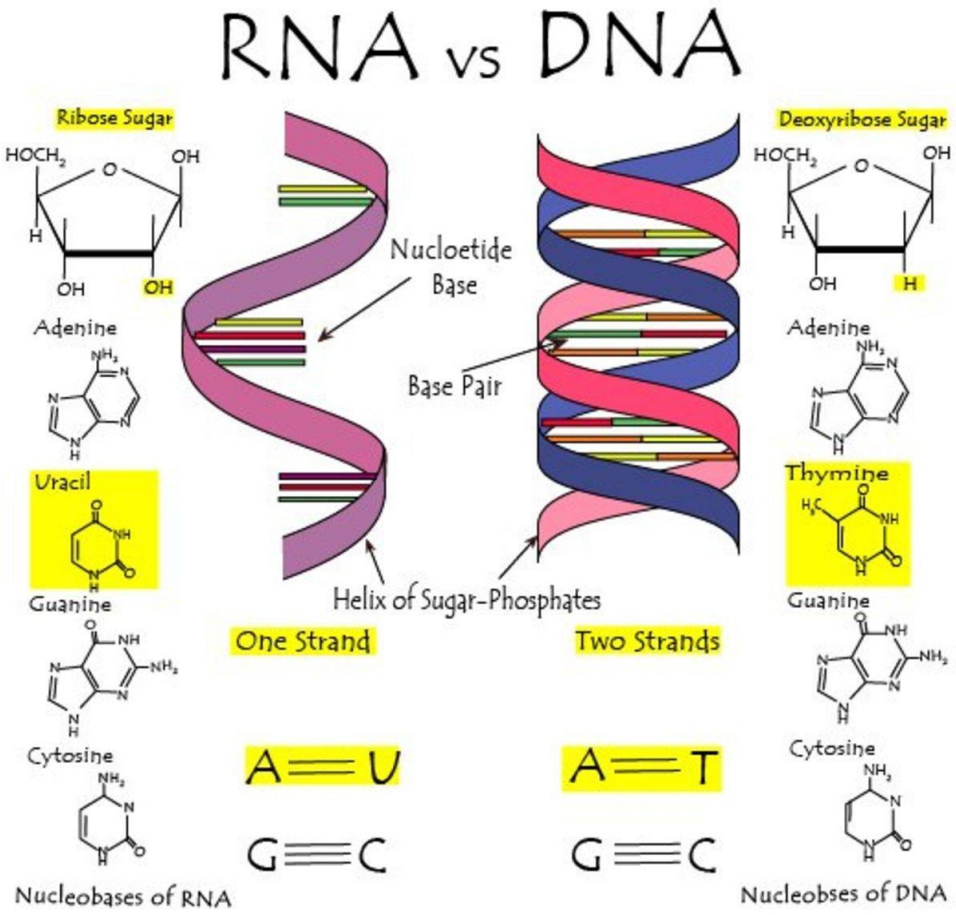

Figure 2: RNA vs DNA [62]

1.3 Protein

Proteins [7,8,9] are the building blocks of life. Proteins are used to build and repair tissues, make hormones, enzymes, and antibodies, help in transport, provide energy, and maintain cell structures. It is a molecule with a long amino-acid chain.

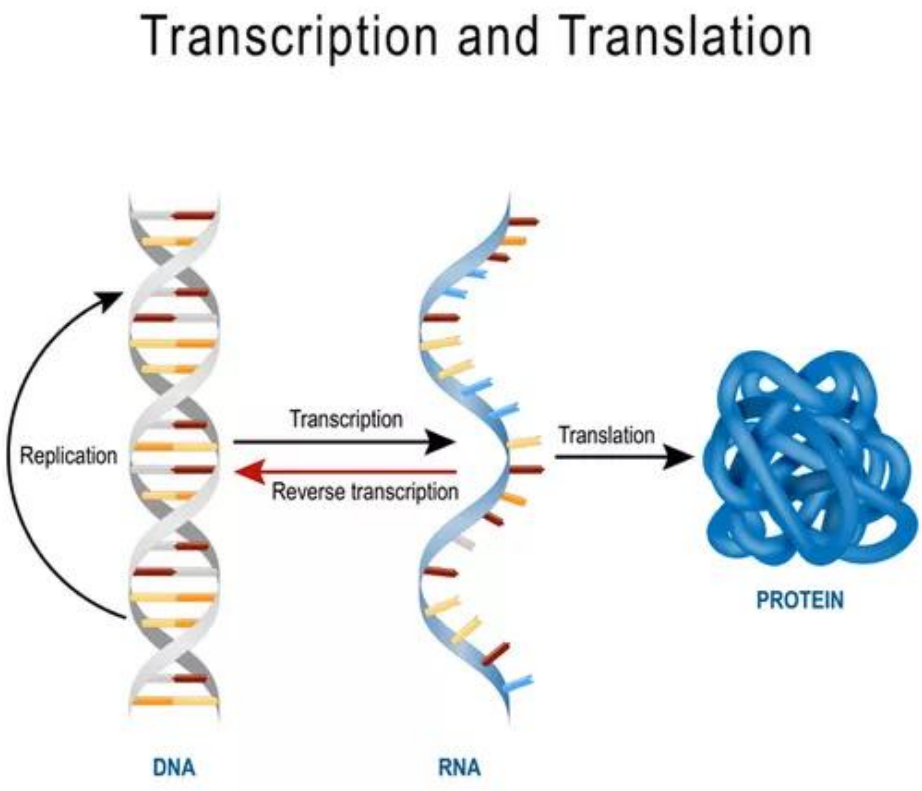

Figure 3: Protein synthesis process [63].

1.4: Central Dogma of Biology

The central dogma of biology [10,11,12] describes the flow of genetic information from DNA to RNA, which is used to form functional protein products.

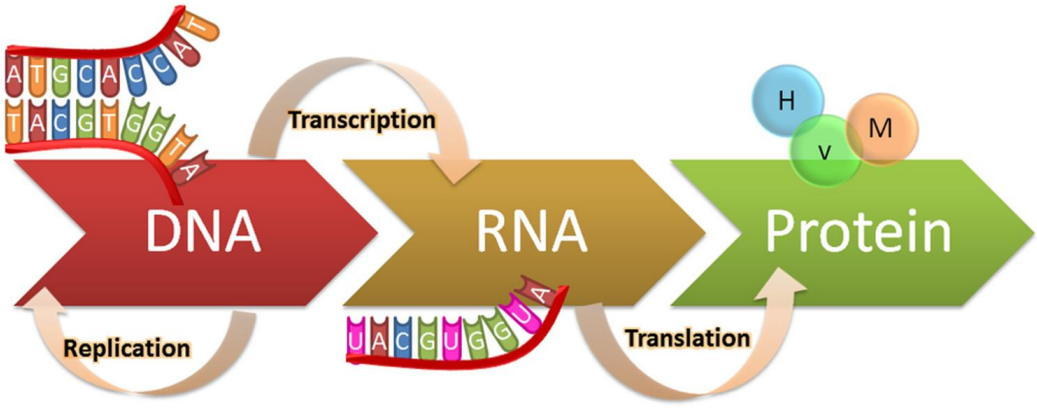

Figure 4: Central Dogma of molecular biology [64]

1.5 **Machine Learning**

Machine learning [13,14,15] is a part of Artificial Intelligence (AI), which learns and adapts

itself using the provided data. It is mainly used for prediction tasks. It is used for weather prediction, classification tasks, pattern recognition, automation, image and speech processing, natural language processing, healthcare, and other tasks. Machine learning is also useful in non-coding RNA research, as a large amount of data are generated by next-generation sequencing (NGS). The application of machine learning in this study has enhanced genomic studies and personalized medicine. Researchers can predict RNA-protein interactions, divide RNAs types, and identify potential treatment targets more precisely using the developed advanced algorithms and large amounts of data. The combination of machine learning with non-coding RNA science is transforming our view of gene regulation and may offer new methods for disease diagnosis and treatment.

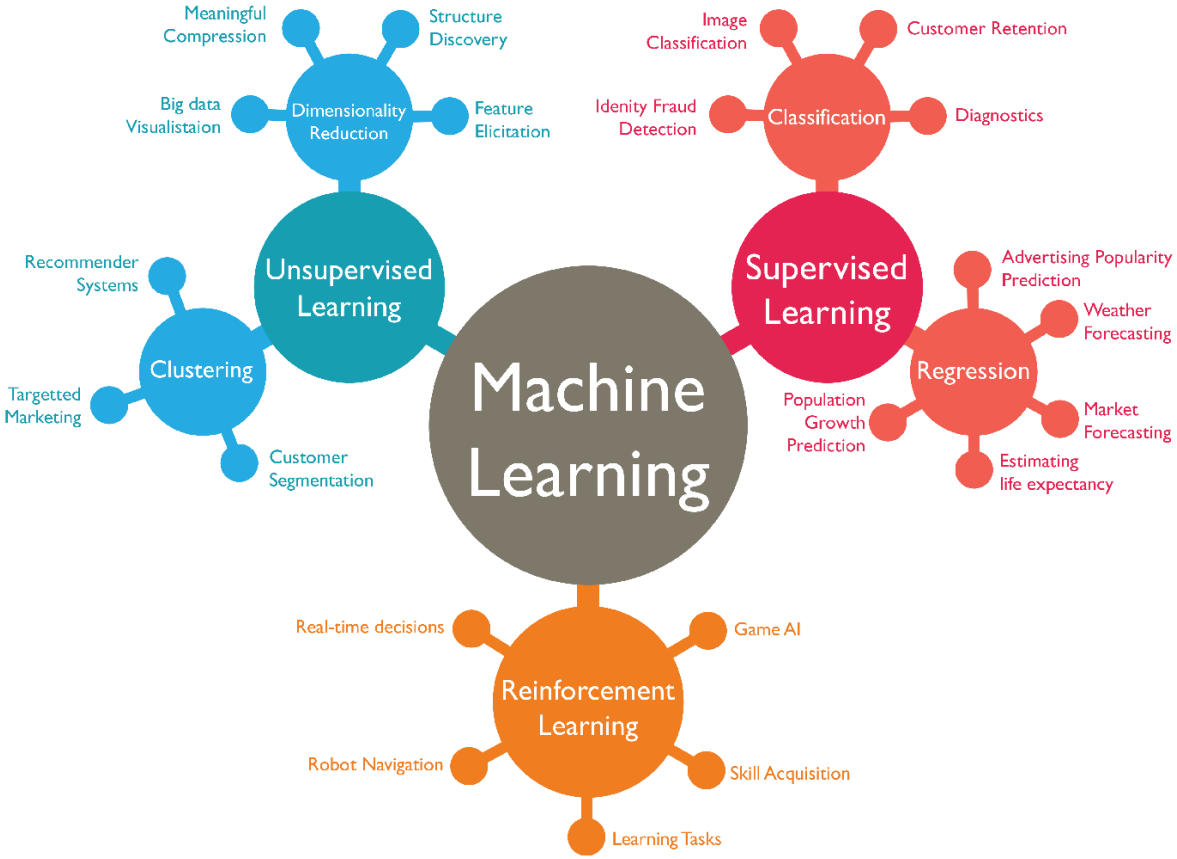

Figure 5: Introduction to machine learning, its kinds, and how it is used in everyday life [65]

1.6 Deep Learning

Deep learning [16,17,18] is a subdivision of machine learning that seeks to develop its own abilities independently of the computer algorithms. This includes algorithms based on brain structure and functionality, which are known as artificial neural networks. These

networks are composed of multiple interconnected layers of neurons and require considerable processing data.

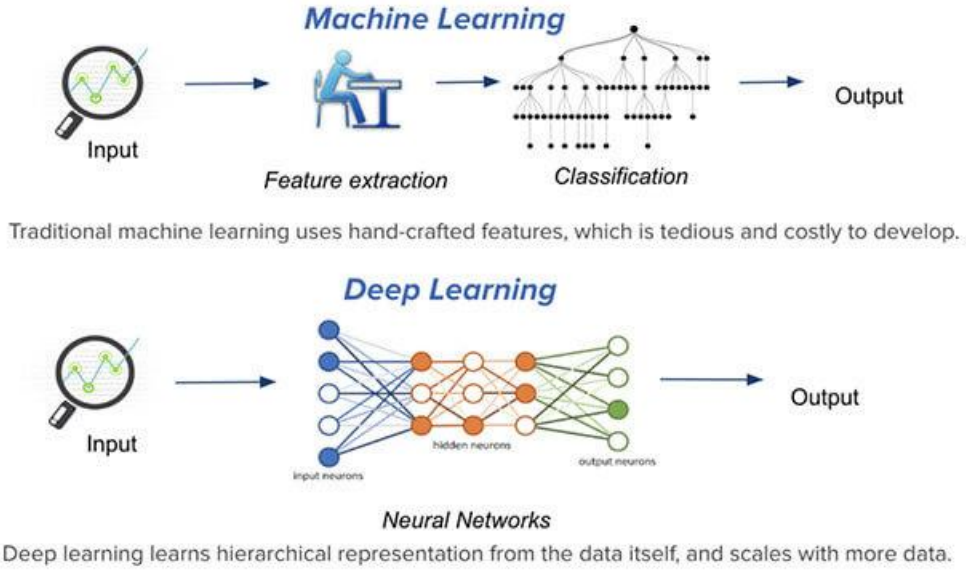

Figure 6: Machine Learning vs Deep Learning [66]

2. Literature review

Non-coding RNAs (ncRNAs) do not code for proteins; however, in their original form, they are responsible for many cellular processes [19, 20, 21]. Recent studies have shown that 93% of the human genome is transcribed [22, 23]. Therefore, creating a better ncRNA classifier would help predict useful ncRNAs and their roles, given the large available gene data sets [24]. The accuracy of the classification depends on the method used by the classifier and the input features selected. Performance metrics help identify the best classifiers by comparing their performance.

Yan Karklin et al. [25] leveraged the geometric and topological characteristics of secondary structures to extract features for classifying non-coding RNA (ncRNA). Jinfengliu et al. [26] utilized protein sequence features to compare protein-coding RNAs with non-coding RNAs. Kong et al. [27] produced a Coding Potential Calculator (CPC) using an SVM classifier. The Frame Finder software was used to identify the longest frames. The feature vector includes three components: log-odd score, coverage of the predicted ORF and integrity of the predicted ORF. Through analysis of the resulting BLASTX search, three primary features were identified: Hit Score, Frame Score, and Number of Hits. Liam et al. [28] used graph-based features as attributes for identifying and classifying non-coding RNA. SVMs were trained using the selected features to distinguish between functional and non-functional RNA, with a Matthews correlation coefficient (MCC) ranging from 0.61 to 0.98, and a sensitivity and specificity of 0.73. Panwaret et al. [29] developed a tool for comparing coding and non-coding RNA and classifying ncRNAs into their respective categories. Single-feature trinucleotide composition was used to train the SVM classifier and the resulting MCC could be said to be 0.98. Chantsalnyam et al. [30] used CNN and ncRNA data to predict ncRNAs. Smith et al. [31] introduced deep learning algorithms to predict ncRNAs. Johnson et al. [32] did comparative study of different deep learning algorithms for prediction of ncRNAs. One study by Wang et al. [33] used deep learning algorithms to predict lncRNAs. The field of deep learning to classify small non-coding RNAs was covered by Chen et al. [34], who provided a hybrid model relying on CNNs and Bidirectional Long Short-Term Memory networks, showing that the latter are more effective in classifying small ncRNAs of different types. Zhang, J., et al. [35] has given an extensive overview of the latest progress in using deep

learning methods on ncRNA classification, on one hand, discussing the challenges, the current trends, and the perspectives of their application in the field. Zhang, J., et al. [36] focused on the functional role of non-coding RNAs having introduced DeepLNC, which predicts the relationship between long non-coding RNAs and diseases, showing a better rate of prediction of disease-associated lncRNAs.

3. Rationale/ Significance of the Study

Non-coding RNA are important in all living organisms. They assist in processes such as translation, RNA splicing and DNA replication. They are also associated with diseases such as cancer, autism, and Alzheimer's disease. Therefore, it is important to identify and classify non-coding RNA using appropriate methods. However, because gene datasets are extremely large, this cannot be performed in laboratories. Therefore, computer techniques must be employed. In this study, we aimed to create new computer methods for identifying and classifying noncoding RNA.

4. Problem Statement

According to the Central Dogma of Molecular Biology, DNA is transcribed into RNA, which is then translated into proteins. However, most RNA do not translate into proteins. These RNAs are known as non-coding RNAs (ncRNAs) and are involved in many biological processes. With the advancement of next-generation sequencing (NGS), a large amount of RNA data has been generated in recent years. Laboratory identification and classification of ncRNAs from large RNA datasets is almost impossible. There is a need for computational techniques that can identify these ncRNAs from the large number of RNAs and classify them into their respective categories. In this study, we will develop a novel computational method to identify and classify ncRNAs.

5. Objectives of Research

RO1. To review the existing literature to identify the present gaps, trends, and computational techniques for the identification and classification of ncRNAs.

RO2. To develop a novel feature extraction method that can extract most of the sequence-based and structural features of ncRNAs very efficiently.

RO3. We aimed to create a novel computational technique that can accurately identify non-coding RNAs from a large number of sequence datasets.

RO4. To develop a novel computational technique that can classify the identified non-coding RNAs in their respective classes

6. Methodology

6.1 Data Collection

Following sources may be used for the collection of data for non-coding RNA research: Rfam 14.7 [37], NONCODE [38], RNAcentral [39], ncRNAdb [40], deepBase [41], LNCipidea [42], lncRNAdb [43], GENCODE [44], EMBL Nucleotide sequence [45], Ensembl Genomes [46], UCSC Genome Browser [47], NPInter v4.0 [48], RefSeq [49] and lncRN

6.2 Feature selection and extraction

Feature extraction and selection are the most important steps in computational techniques, where machine or deep learning algorithms are used. Feature selection methods can be broadly divided into filter, wrapper, and embedded methods, each with its own advantages and disadvantages. Filter methods include correlation-based feature selection and mutual information, which are computationally efficient and do not require a classification algorithm to be applied. Wrapper methods e.g. recursive feature elimination The wrapper method, like recursive feature elimination, uses recursive subsets of features, tested on the selected classifier, possibly with better performance, at the cost of increased computation.

6.3 Training of the model

A wide array of machine learning algorithms is available for tasks such as the identification and classification of objects. Depending on the specific domain, an appropriate machine- or deep-learning algorithm can be selected. In this study, we evaluated the effectiveness of various machine learning algorithms on selected features and determined the algorithm that delivered the most optimal results.

6.4 Testing and hyperparameter tuning

Numerous tools are available for predicting, identifying, and classifying noncoding RNA, including RNAcon [51], CNCI [52], PLEK [53], CONC [54], CPC [55], RNAfold [56], BLAST [57], BLAT [58], CSHMM [59], and RNAz [60]. Our goal was to compare our findings with those of existing tools to evaluate the superiority of the tools we developed.

7. Expected outcome of the Research

This study will introduce a novel approach for studying non-coding RNA using computational methods. This study will propose a novel method for extracting features that captures more useful information from RNA sequences and structures. A new model will be created to identify non-coding RNA more accurately than current tools. Another model will be designed to classify different types of non-coding RNA in a simple and reliable manner. This research is also innovative because it combines these new features and models into a complete system. This system is expected to perform well on different datasets and provide more stable results than existing models. Overall, this study provides new ideas, improved techniques, and a practical framework that can support future research on non-coding RNA.

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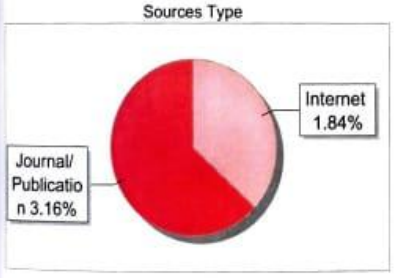

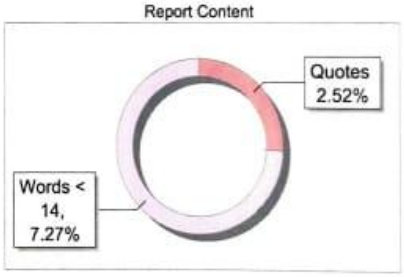

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Manish | Cuma
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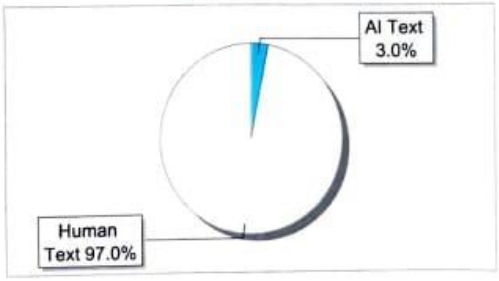

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